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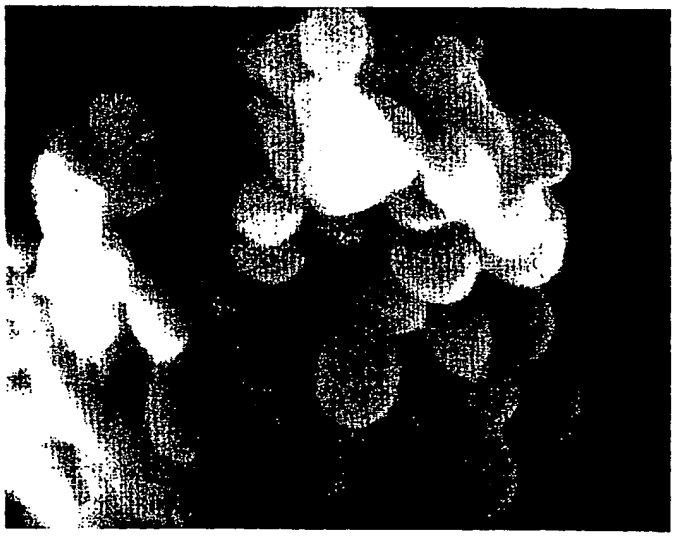
(54) Title: ORAL NANOSPHERE DELIVERY

HEPARIN NANOSPHERES 600-800nm



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(57) Abstract: Oral nanoparticulate pharmaceutical formulations and related methods for controlled release delivery of chemotherapeutic and macromolecular agents.

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ORAL NANOSPHERE DELIVERY

This application claims priority benefit of U.S. provisional application serial number 60/252,070 filed on November 20, 2000, the entirety of which is incorporated herein by reference.

FIELD OF INVENTION

The present invention relates to improved pharmaceutical formulations for administration of macromolecules or drugs not bioavailable using standard delivery platforms. More specifically, the invention relates to pharmaceutical formulations of controlled release rate dosages that may be administered orally using nanospheres or like particles.

BACKGROUND OF THE INVENTION

Oral delivery of drugs or long acting parenteral dosage forms of drugs is preferred by the patient and physician because of improved compliance and the inherent beneficial effect of constant pharmacodynamic action. By themselves, biological macromolecules, e.g. proteins, polypeptides, and some complex carbohydrates, are degraded by enzyme systems in the gastro- intestinal (GI) tract and are thus not orally active. Even if a protein or polypeptide is stable in the digestive tract, absorption into the bloodstream is often restricted. This problem arises from the fact that the drugs are destroyed before being absorbed or they are of such a size and nature that the body cannot absorb the medication. Similarly, their injectable forms have short durations of action, requiring frequent injections, making the products unsuitable for use in the non-hospitalized patient.

Other bioactive molecules, including many cancer chemotherapeutic agents, are not orally bioavailable or are poorly absorbed from the GI tract.

To date, demonstrated oral delivery of biological macromolecules has been limited in terms of the scope of the molecules, the bioavailability achieved, the duration of effective plasma levels and the species in which the effect has been observed. "Nanosphere Based Oral Insulin Delivery" Gerald P. Carino et al.; Journal of Controlled Release, Vol 65, No. 1-2, March 2000. In this work oral delivery of insulin using a biodegradable nanoparticle was demonstrated for rats with a duration of effectiveness of up to 6 hrs. and an oral bioavailability of 11.2 % relative to a subcutaneous injection. Efforts in the art of longer release formulations for oral delivery are also limited. Insulin

was delivered orally to rats in 145 nm nanocapsules composed of isobutyl cyanoacrylate. "Poly(alkyl cyanoacrylate) nanospheres for oral administration of insulin" C. Damge et al.; J. Pharm. Sci. Vol 86, No. 12, pgs. 1403-9, December 1995. In the latter study the insulin effect was absent or lasted less than 2 days unless an oily medium was used to administer the nanospheres, in contrast to the present invention. Furthermore, attempts to obtain effective insulin delivery in humans with cyanoacrylate nanospheres were not successful. Additionally, biodegradable nanospheres were examined for the delivery of heparin. See, "Oral Bioavailability of Heparin Using a Novel Delivery System," James M. Dunn and Alan S. Hollister; Current Therapeutic Research, Vol. 56, No. 8, August 1995; See also PCT Application WO97/04747, February 13, 1997. However, no teaching is provided as to which, if any, combinations or formulation parameters might be administered orally or could be employed to achieve a desired therapeutic effect. The nanospheres are described neither chemically nor functionally. There is no disclosure as to which polymers could be used to promote effective delivery. Even so, stable plasma levels were not evidenced beyond six days and oral delivery of any of the above preparations has not been demonstrated in humans.

SUMMARY OF THE INVENTION

It is an object of this invention to provide one or more delivery systems for oral administration of therapeutic agents and/or macromolecules overcoming numerous deficiencies and shortcomings of the prior art, including those described above. Accordingly, it can also be an object of the present invention to provide a composition and/or method relating to the therapeutic administration, delivery, lymphatic uptake and/or controlled release of such a composition.

It can also be an object of the present invention to provide and optimize various biodegradable components and systems for use in conjunction with nanoparticulate delivery methods for a variety of therapeutic agents, such components or systems as can be modified through use of one or more bioadhesives or various other adjuvants, and such agents as can be administered to mammalian species, including humans.

It will be understood by those skilled in the art that one or more aspects of this invention can meet certain objectives, while one or more other aspects can meet certain other objectives. Each objective may not apply equally, in all its respects, to every aspect of this invention. As such, the following objects can be viewed in the alternative with respect to any one aspect of this invention.

Other objects, features, benefits and advantages of the present invention will be apparent from this summary and its description of various preferred embodiments, and will be readily apparent to those skilled in the art having knowledge of various therapeutic formulations and methods for delivery. Such objects, features, benefits and advantages will be apparent from the above as taken in conjunction with the following figures and all reasonable inferences to be drawn there from.

In part, the present invention relates to nanoparticulates and/or spheres formulated to include a cancer chemotherapeutic agent, such agent as can be used in conjunction with a related method for delivery in patients with cancer and particularly those with metastatic cancer in the lymphatic system. Such nanoparticulates can comprise therapeutic agents and the structural delivery components described herein, as well as those agents and/or components known to those skilled in the art made aware of this invention. The nanoparticulates can be formulated and prepared as described below. Oral administration at predetermined dosage levels can facilitate lymphatic delivery and uptake of those particulates suitably dimensioned. The desired therapeutic effect includes sustained release post-dosage and/or stable plasma concentrations of the subject agent. In addition there is amelioration of the hepatic (first pass effect), whereby the drug is not immediately presented to the hepatic system for degradation. The combination of lymphatic delivery and sustained release can provide a unique benefit in the treatment of various cancers and malignant disease of the lymphatic system. As described below, such delivery and/or sustained release can be controlled by way of nanoparticulate formulation.

In part, the present invention can also include various methods of using nanostructural components to modify the plasma profile of a therapeutic agent post-dosage. Such methods are especially useful for those agents, typically macromolecules, which heretofore have not been available through oral administration. Such methods include providing nanoparticulate rods or spheres formulated for the desired therapeutic effect. As mentioned above, such formulations can include anti-cancer agents, but also macromolecules including, but not limited to, heparin. Regardless, as described more fully below, the structural composition of a nanoparticulate/sphere can be altered to extend controlled release and/or enhance stable plasma levels over time.

In particular, the present invention includes nanoparticulate structural components comprising a therapeutic dose of heparin and a polymeric structural component designed to extend and/or sustain controlled release. In preferred embodiments, the structural component is a co-polymer of lactic acid and glycolic acid. The amount of this co-

polymeric system in the structural component can be used to affect controlled release and thereby modify the plasma profile of heparin post-dosage. In preferred embodiments, the co-polymer is present in amounts sufficiently distinguished over the prior art to provide the surprising and unexpected results described herein.

The present invention obviates the problems of solvation as well as the need for classic absorption for delivery of those drugs where the molecular size is too large to be absorbed when the product is formulated and administered, and/or because of the deleterious effects of gastrointestinal enzymes, and other factors. The invention is directed to pharmaceutical formulations for effective controlled release of many drugs not now orally available. These formulations, properly adjusted, also may be administered by inhalation and achieve similar kinetic characteristics.

The pharmaceutical formulations of this invention are made by entrapping the macromolecule and/or drug of choice in either an organic or water phase biodegradable polymer system to produce nanoparticles. These nanoparticles can, optionally, be coated or combined with one or more bioadhesive adjuvants to promote adherence of the particles and their associated active material or medications to the intestinal wall.

Two pharmaceutical formulation embodiments can be used to form the nanoparticles: In the first instance, drugs that are rugged and can withstand organic solvents are treated by entrapment in single or combinations of biodegradable polymers. Alternatively, non-organic solvents may be used, but it is preferred to entrap drugs that need a water-based system, with secondary attachment to a cyclodextrin. This entrapped drug nanoparticle complex may then secondarily be entrapped in liposomes.

With those drugs that are delicate to changes in their environment, the drugs can first be entrapped into a cyclodextrin for protection, and then granulated with water soluble or organic soluble polymers. The invention is therefore suitable for using both organic and non-organic solvents.

An advantage of the nanoparticulate/sphere system can be, under appropriate conditions, absorption by the lymphatic system. With use of a resistant coating, the enzymes or degradative influences present in the gastrointestinal tract do not affect such a system. Bioadhesive polymers can ensure a more prolonged duration of time in which the nanoparticles are in contact with the intestinal mucosa, reducing the deleterious action of heightened gastrointestinal peristalsis. All of these compounds work in harmony to produce viable products that have demonstrated favorable and reproducible effects in animals.

The invention thus describes simple and predictable methods for the preparation of oral or inhalation dosage forms of a macromolecule, drug and/or therapeutic agent in biodegradable polymers. The following combinations are representative of those, which can be used to formulate such a controlled release orally administered pharmaceutical product, in accordance with this invention:

A controlled release pharmaceutical formulation with a biologically active molecule which is formed into nanoparticles with biodegradable polymers and then coated with one or more bioadhesive adjuvants.

A controlled release pharmaceutical formulation with a biologically active molecule entrapped in a cyclodextrin, which is then formed into nanoparticles with biodegradable water-soluble polymers, and then coated with one or more bioadhesive adjuvants.

The sphere size in the nanoparticle will typically be in the range of 100nm to 2.0 microns, so that the active medication entrapped, may be administered orally or by inhalation. The exact diameter of the nanoparticles may not be critical in some instances, but is sufficiently small for cell diffusion by the lymphatic system.

The rate of active drug release from the entrapped spheres is dependent upon the basic kinetics of the drug administered, the amount of polymer, the cyclodextrin used and, in the case of parenteral administration, the method of administration, the area of deposition, and the vascularity of the body region. With inhalation dosing, it is not necessary to use the bioadhesive adjuvants. The larger the size of the particle, 1-5 microns, has been demonstrated to be the most efficient size for inhalation delivery of drugs in biodegradable microspheres.

The invention is thus directed to controlled release rate formulations of biologically active molecules, especially macromolecules. Any therapeutic drug, biological active protein or polypeptide, polysaccharide molecule, and the like, referred to generally herein as "bioactive molecule", "bioactive material," "biologically active molecule," or "drug," can be formulated according to this invention. In addition, more than one drug can be formulated together according to this invention.

As has been previously noted, poly(d,l-lactide-co-glycolide) (PLGA) nanoparticles are used for controlling the release rate of drugs from their matrix. These products are used for several reasons.

1. They are biodegradable and form non-toxic monomers.

2. The release kinetics of the active moiety can be controlled by varying the amount and nature, as well as the molecular weight and inherent viscosity of the polymer or polymers used.

3. The polymer matrix protects proteins and peptides against the destructive conditions observed when these products are given orally.

However when proteins are incorporated in these matrices problems occur such as reduction of bioactivity and poor entrapment. This may be due to the harsh chemical and mechanical stress imposed upon the protein during the manufacturing processes. By the addition of protein stabilizing agents these problems may be reduced. In order to improve retention of bioactivity, sucrose, mannitol, poloxamer 407, may be added.

Even though entrapment efficiency may be high, addition of these sugars and hydrophilic compounds may increase the entrapment rate significantly. It is known that the sugars stabilize the proteins by preferential exclusion, subsequently the proteins interact more strongly with water than with solvents. The amount of sugars added to the mixture, will depend upon the product, but in general are in the range of 3%-15% by weight.

Primary candidates for development into controlled release oral formulations are the widely used drugs, particularly those drugs with low bioavailability and drugs currently only capable of being administered via parenteral means. These include, but are not limited to, therapeutic proteins such as insulin, GM-CSF and G-CSF, erythropoetin, interferon alpha, interferon beta, interferon gamma and other cytokines, IL-2 and other interleukins, antibodies, and peptide hormones. Other candidates for oral controlled release delivery of the invention include antiviral agents, antibacterial agents, antifungal agents, and fertility hormones. One such pharmaceutical that can be prepared according to this invention is heparin, a classic large molecular weight drug that has been considered the gold standard for effectiveness of new drug delivery systems for large polypeptide or carbohydrate drugs, and has previously been unavailable by oral means for controlled release over sustained periods. With the pharmaceutical formulations of this invention, these drugs are not only absorbed orally, but their pharmacological effects are prolonged.

Heparin is a well-established drug that is a complex polysaccharide that is highly charged (electronegative). It has a molecular weight of 20,000–60,000 daltons, and is covalently attached to a core protein found in most secretory cells. Heparin is a multifaceted drug with primary actions as an anticoagulant. When venous or arterial thrombi occur and the patient survives, heparin is given at doses of 10,000-20,000 units

every 4–6 hours to maintain blood levels at 0.5-1.5 anti-factor Xa units/mL, which should prevent thrombus enlargement and alleviate the possibility of embolic phenomena. After this, the heparin dose is usually maintained at 10,000 – 15,000 units every 4-6 hours to prevent further thromboembolic events.

In addition, heparin has the ability to bind to the arterial wall following angioplasty and ameliorate the proliferation of smooth muscle cells and ameliorate the restenosis that so often occurs with this procedure. Also, heparin is used as a preventive agent for those patients that are at close risk of stroke or heart attack as well as the patients recovering from a heart attack. Heparin also has a clearing effect in the blood by activating lipoprotein lipase on the cell surface. This action clears hyperlipoproteinemia and lowers the low-density lipoprotein. In animals, it has reversed the athrogenic deposits on the arterial walls, which is a phenomenon of arteriosclerosis in humans.

In accordance with several other aspects of this invention, the following compounds can also be used in conjunction with a nanodimensioned system for sustained release oral delivery: such compounds and/or materials including but not limited to anticancer drugs or chemotherapeutic agents, including 5-fluorouracil, paclitaxel and covalent derivatives, docetaxel, taxotere, doxorubicin, daunorubicin, epirubicin, methotrexate, leucovorin, cisplatin, carboplatin, cyclophosphamide, vincristine, vinblastine, vinorelbine, BCNU, CCNU, camptothecin and covalent derivatives, irinotecan (CPT-11), lurtotecan, 9-nitrocamptothecin, lipophilic camptothecin BNP1350, 9-methoxycamptothecin, topotecan; monoclonal antibodies and antibody fragments; anti-VEGF antibody and fragments, anti-VEGF aptamer, angiostatin, endostatin and other anti-angiogensis agents; aptamers for anticancer targets such as anti-tenascin aptamer; somatostatin, and peptides for anticancer therapy.

Nanospheres/particulates may be produced by methods known in the art. Typically, the active medicinal agent is dissolved in a suitable solvent such as buffered water or purified water which may contain a surfactant. The biodegradable polymers are most often dissolved in organic agents such as dichlomethane, acetone, ethyl acetate and the like or combinations of these solvents. The aqueous solution of active is then combined with the organic phase with rapid stirring to form a water in oil emulsion. This emulsion is then dispersed in purified water or buffer, typically including a surfactant. This solution is stirred, homogenized or sonicated to form nanoparticles or nanospheres in the desired size range. Isopropyl alcohol may be added to harden the nanospheres at this point. The organic solvent may be removed from the nanosphere emulsion by reduced

pressure or by addition of the solution to a volume of water in which the solvent is partially soluble, and the nanospheres recovered by filtration. Alternately, the emulsion may be dried completely and the product recovered. Spray drying may be employed to remove the water and organic solvent to produce a dry powder.

Hydrolysis of the polymer is the most desirable breakdown mechanism since this will produce low molecular weight by-products. Biodegradable polymers include: poly (lactic acid), poly (glycolic acid), poly (d,l-lactide-co-glycolide) (poly (epsilon-caprolactone), poly (3-hydroxybutyrate), poly (3-hydroxyvalerate), poly (ortho esters), polyanhydrides, dextran, cross-linked polyvinyl alcohol, polyhydroxy methacrylate, polycyanoacrylates, poly (phosphoesters), poly (hydroxy butyrate co-valerate), poly (2-hydroxyethyl glutamate), polyvinylpyrrolidone, poly (ethylene glycol), poly (propylene glycol), chitosan, pullulan, zein, alginic acid and alginate salts.

In addition, other methacrylate polymers can be used specifically in water-soluble systems. These include, but are not limited to acrylic acid, methacrylacetic cyanoethyl methacrylic, aminoakyl methacrylate copolymers, ethoxyethyl methacrylate copolymers, polymethacrylic acid, methacrylate acid alkylamide copolymer, polymethacrylic acid, polyacrylic acid, methacrylic acid alkylamide copolymer, polymethacrylic acid (anhydride), and polymethyl methacrylate. The advantage of the acrylate series is that when properly compounded they are water-soluble or can be supplied as a 30% aqueous solution with varying degrees of solubility.

Other polymers known to the art can be used in either the water or organic phase, if properly prepared. An example of this is alginic acid in intimate admixture with the drug, then cross-linking the alginic acid with known inorganic elements such as divalent ions of zinc, magnesium, calcium, or sodium salts. Likewise pectin, zein, and guar gum can be used and cross-linked with a metallic ion. Ethyl cellulose can also be used as a polymer to entrap the molecule and can be used in an aqueous or organic solvent system. Similar examples are polyvinyl acid phthalate, cellulose acid phthalate, cellulose acid trimaleate phthalate and similar cellulosic polymers such as hydroxypropyl methylcellulose phthalate and the like.

Cyclodextrins are a class of compounds derived from corn. They are cyclic, non-reducing oligosaccharides built up from six, seven, and eight glucopyranose rings known respectively as alpha, beta, and gamma cyclodextrins. In addition, hydroxypropyl and other groups have been and are added to the molecule giving each series its own specific characteristics and pharmacological behavior. The cyclodextrins act as a

biodegradable trapping agent, incorporating the active medication into its core and forming a unique molecular inclusion complex. These complexes provide an anchoring compound without the formation of covalent bonds. This guest-host complex protects the active drug, while not forming tight chemical bonds, and the present invention demonstrates its usefulness in extending the release of drug from the nanospheres.

With the drug in a buffer or suitable solution and, with agitation, cyclodextrins can be used to entrap the drug, providing a guest-host interaction. The type of cyclodextrins used will again depend upon the molecule and the degree of protection required. These parameters can easily be determined by routine experimentation by one of skill in the art.

Preferred bioadhesive adjuvant(s) that can be added to formulations of this invention include hydroxypropyl methylcellulose, methylcellulose, pectin, guar gum, xantham gums, gum acacia, gum dragon, hydroxypropyl alginate, sodium carboxymethyl cellulose, carbomers, acrylic acid derivatives and those of similar pharmaceutical characteristics and behavior. These adjuvants are pharmacopeial items and are blended in with the nanoparticle granulate at the final stage of production. The preferred dose is a single or combination of these adjuvants at a 50/50 W/W ratio and a percentage weight of the total granulate of 0.1-3% preferably in the range of 0.3-2.0% and most preferably 0.2-1.2% weight percentage.

Without restriction to any one theory or mode of operation, the enhanced results and/or therapeutic activities demonstrated by the present invention can be attributed to one or more of several factors not specifically disclosed, taught or suggested by the prior art. Such factors include but are not limited to nanoparticulate formulations incorporating a cyclodextrin component, the benefits of which are illustrated in several of the following examples. Other such factors include heat treatment or drying of the nanoparticulates after incorporation of the desired therapeutic agent, and the concentration of such an agent relative to the nanoparticulate matrix, with lower relative active concentrations providing extended therapeutic levels as compared to the prior art. These and other factors are illustrated in the context of several representative formulations, but are applicable with comparable effect to various other formulations and aspects of this invention.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A and 1B are photomicrographs of the nanospheres of Examples 7 and 8, showing clearly passage through lymph ducts and into a lymph node, respectively; Figure 1C shows nanospheres adhered to the intestinal wall. The nanospheres were stained with fluorescein.

Figures 2A and 2B are photomicrographs of heparin nanospheres (600-800 nm), the spheres prepared in accordance with this invention and the micrographs taken as shown.

EXAMPLES OF THE INVENTION

The following examples describe the preparation of several novel nanoparticle formulations of the present invention and their administration (e.g., orally) to achieve delivery of a variety of therapeutic agents *in vivo*, particularly to the lymph system. In particular, as described below, the present invention enables for the first time the oral delivery of therapeutic agents *in vivo* at significant levels and duration, such that the agents reach the lymphatic system using nanoparticle formulations which include only biocompatible components safe for use in humans. Embodiments of the present invention, in addition to those illustrated, will be readily understood by those skilled in the art and are included within the scope of the invention, including novel processes and products derived from the invention whether as individual features or in combination with each other to produce novel combinations. The invention is illustrated further by the following examples, which are not to be taken as limiting in any way.

The following non-limiting examples and data illustrate various aspects and features relating to the formulations/compositions and/or methods of the present invention, including the use of nanoparticulate materials for the delivery of various macromolecules and/or therapeutic agents. In comparison with the prior art, the present methods and formulations/compositions provide results and data, which are surprising, unexpected and contrary to the prior art. While the utility of this invention can be illustrated through use of several polymeric systems and active agents, it will be understood by those skilled in the art that comparable results are obtainable with various other formulations, compositions and therapeutic agents, as are commensurate with the scope of this invention.

In particular, Examples 1 and 2 illustrate various aspects of the present invention, as can be described or inferred from the concentration of heparin in rabbit plasma over

time after oral dosing with heparin-containing nanospheres suspended in a bioadhesive adjuvant. The nanospheres comprised a 50/50 (w/w) mixture of heparin and a biodegradable polymer poly(d,l-lactide-co-glycolide), PLGA. In addition the formulation contained beta cyclodextrin in the aqueous phase. This formulation is in contrast to the prior art where the polymer composition was 33% poly(d,l-lactide-co-glycolide) and 67% poly(3-hydroxybutyrate-co-valerate) without beta cyclodextrin (ref. "Oral Bioavailability of Heparin Using a Novel Delivery System" (1995) Current Therapeutic Research 56, pgs. 738-745). In the present study two rabbits each in three dosage groups had plasma samples taken over the course of 23 days after dosing. In the earlier published study one dose level was administered to 12 rabbits and the plasma levels determined out to 144 hrs post dosing. Significant differences between the two studies in the plasma level profile over time and in the maximum levels achieved point to important and/or critical formulation variables, such including but not limited to an increase in the amount of PLGA and the addition of PVA. The use of PLGA-only formulations, compared to formulations in the prior art using other polymers (either alone or in conjunction with PLGA), is noteworthy. PLGA-only formulations have several advantages including a history of use in humans (as injectable dosage forms). In addition, the use of PVA is an advantage over the prior art, which doesn't disclose use of a surfactant. Use of a surfactant can provide physical stability of the emulsion during the process.

Example 1

Heparin Nanospheres. Nanospheres were formed from 1:1 (w/w) poly(d,l-lactide-co-glycolide) and heparin with the emulsion prepared in an aqueous solution of beta-cyclodextrin and polyvinyl alcohol. Scanning Electron Microscopy showed aggregates made up of smaller, roughly spherical bodies with diameters in the range 500-800 nm. Materials and a method for preparing nanospheres are detailed in Example 2, below.

Doses of 200mg/kg, 400 mg/kg and 600 mg/kg (based on weight of the heparin nanosphere granulate) were administered by oral gavage in aqueous bioadhesive polymer adjuvant solution to 2 rabbits at each dose level. Plasma was sampled at intervals up to 552 hrs following the single dose. Plasma heparin levels were determined using a factor Xa chromogenic assay with a quantitation limit of about 0.1 U/ml. For the 600 mg/kg dose, a therapeutic level (0.39 factor Xa units/ml) was achieved at 2 hrs post dosing. The

levels declined thereafter (day 4; <0.1 U/ml) until rising at day 6 through 10 to values between 0.40 and 0.42 U/ml. Plasma heparin declined to unquantifiable levels after day 10. The lower doses produced a roughly similar pattern of increase of plasma heparin levels between day 6 and 10.

The plasma level dynamics for the present heparin nanosphere preparation is in contrast to that in the prior art where the heparin levels did not reach full therapeutic values by 2 hours, and the high levels that were reached by one day were tapering off at 6 days when the study was concluded.

The present example illustrates the ability to achieve significant heparin plasma levels by 2 hrs post dosing, and to sustain levels to 10 days. Such a result was achieved in part by adjusting the relative amount of PLGA in the preparation and the use of PVA. No guidance is available from the prior art as to how to achieve these effects.

Example 2

Heparin Nanosphere Preparation. The following provides another heparin nanosphere preparation of the sort, which can be used to prepare other compositions of this invention. Specifically it provides a preparation which includes the heparin nanospheres described in Example 1.

Product	Lot	Amount	Percent
Distilled Water		200 mL	
Beta cyclodextrin	D-6196-345	200 mg	4.55%
Polyvinyl alcohol (PVA)	44042	200 mg	4.55%
PLGA 50:50 RG 503H	640662	2,000 mg	45.45%
Heparin Powder	30K0537	2,000 mg	45.45%
Isopropyl alcohol	HPLC	10 mL	
Methylene Chloride	HPLC	40 mL	

1. Disperse PVA and Beta cyclodextrin into water and stir at high speed.
2. Into 40 mL of methylene chloride add PLGA 503H; after the PLGA is completely dispersed, add 2000 mg of heparin powder.

3. Pour the organic mixture of PLGA and heparin into the water and blend at high speed. A milky emulsion will form. Then add 10 mL of IPA to harden the spheres and blend at medium speed for 5-7 minutes.
4. Pour mixture into Pyrex plate and heat at 60°C until dry.
5. When dry scrape the dried material from plate completely, and weigh to determine yield. Then pass through #25-26 screen to reduce the size of the particles.
6. Check the powder before and after dissolution with CH_2CL_2 with protamine.

Note weight after immediate scraping = 4.40 gm then after mortar and pestle the weight was 4.678 gm.

Example 3

Nanosphere Preparation. This example illustrates the production of another nanosphere formulation of the present invention, which has an advantage over the prior art in that it involves entrapment of a biological macromolecule (heparin) in a nanosphere using a water-based procedure (compare to prior art in which Red-Lake dye, not a biological macromolecule, was used). The agent to be entrapped in the nanosphere may be sensitive to organic solvent in which case a water/water method can be utilized. This involves dispersion of a water-soluble agent in a suitable fluid media and producing cross linking of the agent, to form microparticles. One such method is the use of alginic acid (1-5%) in a suitable amount of water, blended with a high shear mixer until there is complete dispersion of the alginate. With constant stirring at medium speed calcium chloride solution is added (1-8%) until distinct bead formation occurs. The speed of blending is increased to a maximum rate which produces smaller size particle, i.e.: nanoparticles. These beads are blended at high speed for approximately 8-10 minutes and at the end of the time, 6mL of isopropyl alcohol is added to the mixture to harden the beads. The beads are then dried in vacuo and examined for size and structure, by a scanning electron microscope. After the beads are dry they are then ready for use.

Water-based entrapment of heparin was accomplished by the following method. Beta cyclodextrin was added to 20 ml of purified water and medium heat was applied with continued stirring, until the solution was clear. To the stirring solution was added 500mg of hydroxypropyl methylcellulose K4MP, stirred until clear. Then 500mg of sodium

dextran was added and stirred until clear. Lastly 2mL of ethyl acetate was added with 5mL of glacial acetic acid and 500mg of chitosan with constant vigorous stirring. After all these agents were well blended 1,000mg of heparin was added and blended into the solution. This material was then poured into 200mL of deionized water with 200mg of polyvinyl alcohol, well dispersed in the solution. The shear rate was set to maximum and the water/water emulsion took place and spheres could be seen forming under an optical microscope. Stirring was continued at maximum speed for 7-9 minutes than poured into a Pyrex pan at 60°C. This was allowed to dry overnight and when dry, after 20 hours, scraped from the pan with a razor blade. The fine powder was reduced in size. In water after vortexing the addition of protamine produced only a faint white cloudy discoloration. The addition of dichloromethane and acetone produced only slightly more evidence of a white precipitate. This water method produced a very tight bond and showed in vitro a very slow release of heparin from the nanoparticle matrix.

Example 4

Alternative formulation for heparin nanospheres.

Ingredient	Amount	Wt. Percent
Purified Water	80mL	
Methanol	20mL	
PVA	0.1	4.76%
Heparin Powder 157U/mg	1.00gm	47.62%
Zein	1.00gm	47.62%
	2.1 gm	100.00%

According to the formulation of this example: zein, a prolamine of corn and heparin are placed into 100mL water and methanol. Stir at high speed until opalescence occurs, check for bead formation under microscope. When material is completely stirred, add into Pyrex® pan and heat at 60°C for 24 hours. Scrape the material from the plate when dry and weigh; then check for reaction of excess with protamine. The final weight was 1.97g, a yield of 93.81%.

Example 5

Alternate formulation for heparin nanospheres.

Ingredient	Weight	Percent
25mL Dichloromethane	25gm	
25mL Acetone	25gm	
PolyL-Lactic Acid	300mg	6.82%
Poly3OH Butyric Acid	600mg	13.64%
Poly DL Lactide	300mg	6.82%
Aqueous Heparin 400,000 units	3200mg	72.73%
		100.00%

The heparin nanosphere formulation of this example was prepared by dissolving the polymers listed in the table above in acetone and dichloromethane. When particles were completely dispersed, aqueous heparin was added until mixed. The mixture was then poured into 100mL of aqueous 0.05M KH_2PO_4. The mixture was stirred until an emulsion formed, then turned at high speed; spherical particles were seen by optical microscope. A scanning electron microscope showed nanospheric formation.

Example 6

Improved Method for Formation of Heparin-Containing Nanoparticles.

Ingredients		mg
1. Poly(3-hydroxybutyrate-co-3-OH-covalerate)	80:20	600mg
2.Poly(d,l-lactide co-glycolide)	70:30	300 mg
3.Heparin Sodium 157U/mg		300 mg
4. 400 mL of 0.05M KH_2PO_4, pH 6.8		
5. Polyvinyl Alcohol		100 mg

HPLC quality dichloromethane, (50mL) and HPLC quality acetone (50mL) were added into a 150mL glass beaker with a magnetic stirring bar. The polymers shown above were dissolved in this mixture and separately the heparin was dissolved in 10 ml water. The heparin solution was added to the polymer solution, and the mixture was slowly added to the phosphate buffer in a 800 mL vessel to which has been added the polyvinyl alcohol. During the addition of the solvents the Silverson® mixer was run at low-medium speed. As particles started to form the fluid turned a milky white. At this point the speed was turned to high levels and a drop of the solution was taken out and examined under an optical

microscope. At the first examinations large particles were seen in the solution and within 5-7 minutes at high speed smaller dark particles appeared. The solution was then poured into a Pyrex® plate and placed in an oven at 60° for 24 hours or until the surface was dry. The material was then removed with a razor blade and passed through a 100 mesh screen forming a fine powder to which the adjuvants were added.

In a similar fashion the polyvinyl alcohol can be added to the organic phase if there is an obvious separation of materials.

The yield was 742 mg out of a theoretical yield of 1,400 mg. The resultant powder/granulate was weighed and titrated against protamine in normal saline to ensure that heparin was in fact entrapped in the spheres. To do this, the beads were suspended in normal saline and agitated by vortex. Protamine was then added to the solution and no precipitate was observed. The nanoparticles were assayed before degradation and found to have 0.75 U/mg of heparin.

Acetone and methylene chloride (2 mL) were added to disrupt the beads and, upon vortex, a heavy white paste precipitate was formed. The dry granulate thus produced was then evaluated and quantified.

To facilitate adsorption, the heparin-containing nanoparticles were blended with bioadhesive adjuvants. Nanoparticles were coated by dispersion in 20-mL of an aqueous adjuvant containing 0.5% Carbopol-934P and 0.5% hydroxyproplylmethylcellulose.

Example 7

Lymphatic uptake. Preferred nanosphere preparations of the invention are formulated to enhance lymphatic uptake and increase periods of stable plasma concentrations.

As described below (e.g., in Example 11) such nanosphere preparations can be used for lymphatic delivery of a variety of therapeutic agents, such as chemotherapeutics. The results provided illustrate (with the corresponding figures 1A, 1B and 1C) lymphatic delivery and uptake of the sort efficacious in the context of various other therapeutic agents. Nanoparticles containing fluorescent stains were prepared and administered orally with concomitant bioadhesive adjuvants, as a single dose to anesthetized rabbits, via a gastric tube. The rabbits were sacrificed 7 and 14 days after oral administration of the nanospheres. Both utraviolet light microscopy and direct vision revealed dye-containing spheres widely distributed throughout the animals' bodies.

Nanoparticle formulations comprising polystyrene have been shown in the prior art to be taken up in the lymphatics after oral administration. However, these formulations have not demonstrated delivery of a therapeutic agent for diseases involving the lymphatic system. Moreover, the prior art does not teach as to the need to achieve lymphatic delivery for orally administered formulations in order to achieve the long term controlled release effect demonstrated by the present invention. It should also be noted that polystyrene oral formulations have safety concerns for use in humans due to biocompatibility issues. The present formulations of the invention, which are made with PLGA and other non-toxic materials, demonstrate lymphatic uptake and long term controlled release of a therapeutic agent with a therapeutic effect on a disease involving the lymph system.

Lymphatic uptake has also been demonstrated for microparticles of PLGA in the size range 1 – 10 micrometers. These formulations were shown to be advantageous for generation of an immune response to the encapsulated agent. Such an outcome is counter to the aims of the present invention wherein nanoparticles in the size range 100 nm to 2 micrometers are employed in one embodiment for delivery of therapeutics via lymphatic uptake and treatment of a disease involving the lymph system.

Example 8

Nanosphere Absorption. A number of factors influence the absorption of the nanosphere from the gastrointestinal tract. The primary and most important element is the size of the nanosphere. It has been demonstrated that microparticles in the small intestine should be restricted to a size of less than 10 microns and preferably less than 5 microns. Kinetic studies in the prior art regarding the fate of microsphere within the gastrointestinal lymphatic tract (GALT), demonstrated that particles larger than 5 microns were not transported in the efferent lymphatics, while particles smaller than 5 microns were readily transported through the lymphatic system to the lymph nodes. The present invention relates to nanaospheres having a size smaller than 2 microns and larger than 100 nanometers, as demonstrated by the photomicrographs of 800nm fluorescent stained spheres. These photomicrographs resulted from an *in vivo* experiment, where the spheres were placed with adjuvant into a rabbit ileal pouch and serial sections taken of the spheres progress, thorough the lymphatic pores down the lymph duct and into the lymph nodes. Reference is made to Figures 1A and 1B. The particles of larger size are maintained on Peyers patches in the intestine. (Figure 1C). Most importantly material entrapped in the

nanospheres are distributed throughout the reticuloendothelial system before reaching the vascular highway.

Example 9

Adjuvants. A number of adjuvants were evaluated for mucoadhesion and use with the macromolecules and agents described herein. The strongest to the weakest of a few of the more prominent agents studied were carbopol <alginic acid <xanthan gum <pectin <cellulose gums. A combination of carbopol 974-P and methocel K4MP in pH 6.8 0.05M monophosphate buffer as the adjuvant also was used in preparing several nanosphere preparations of the present invention; for instance, in 10% carbopol 974-P, 10% methocel K4MP, suspended in buffer as described, or formulated into capsules or tablets as the dry adjuvant in combination with a bioactive molecule.

Example 10

Other Nanosphere Preparations. Nanospheres of the present invention can be formulated using the therapeutic agents, polymers and other structural components described herein, such as those listed in the table below, or by using other techniques and structural components known in the art.

Agent	Component
a) 5-fluorouracil	polylactic acid
b) 5-fluorouracil	PLGA
c) doxorubicin	zein
d) carboplatin	dextran
e) methotrexate	chitosan
f) methotrexate	polyvinylalcohol
g) cyclophosphamide	hydroxyvalerate
h) vinblastin	PLGA
i) vinblastin	alginic acid/alginate
j) endostatin	Pullulan

Example 11

Formulation of 5-fluorouracil (5-FU) Nanospheres and Oral Administration to Humans with Cancer. This example demonstrates oral delivery of chemotherapeutic agents to the lymphatic system at sufficient levels and duration for the treatment of

metastatic cancers in humans. The prior art offers no guidance with respect to how to achieve this therapeutic goal.

5FU Nanospheres

Ingredient

1. 400 mL purified water
2. 100 mL Dichloromethane
3. 100 mL Acetone
4. 20 gm DL Lactide Resomer 202 (PLGA)
5. 400 mg 5FU In Deionized Sterile Water

In a Silverson® mixer, organics 2+3 were blended and Resomer was added with moderate speed. The mixture was blended, until the solution was clear. Water was then added with 5FU dispersed into it and stirred on a magnetic stir plate. The mixture was poured slowly into organic or oil phase and continued to be blended until there was an even milky color to the fluid. The mixture was then placed into an oven at 60°C and maintained for 24 hours or until completely dry. The mixture was then removed with a razorblade until all powder was off the plate. The mixture was then bottled and set aside.

Powder Portion 5FU

Ingredients	mg	%
1. Chondroitin Sulfate	200.66	24.73%
2. PEG 3350	201.10	24.78%
3. Starch 1500	35.50	9.37%
4. 5FU Nanospheres	11.83	2.91%
5. Myverol 6000	5.92	0.73%
6. Beta Cyclodextrin	25.00	3.08%
7. Carbomer 974-P	50.00	6.16%
8. L30 D-55	75.00	9.240%
9. L-100 Powder	25.00	3.05%
10. Magnesium Sterate	1.00	0.01%
Total	631 mg	100%

After the nanospheres were made, the following formulation was prepared as a tablet matrix. Items 1-5 in the table above were blended and granulated with a small amount of beta cyclodextrin. Carbomer 974 –P was then blended into the granulate. The

acrylate polymers were blended together and granulated over the rest of the powder. When the granulate was hard and dry after being dried in a fluid bed dryer, magnesium stearate was added as a flow agent and a lubricant. The material was then put through a granulator at size 093 and pressed into tablets weighing 631mg with a hardness of 10-15kg. The disintegration is generally over 4 hours or more.

After checking for dissolution and release rate, the preparation was given to a patient with cancer and titrated up to tolerability or definitive clinical response. In particular, the preparation was given to one 37 year old white male with recurrent metastatic carcinoma of the testicle. He had metastasis to the lung, brain, and also had lymphatic enlargement. The physician treating the patient said he had about 2 weeks to live.

The patient started on the tablets without complication, taking 4 tablets twice a day. He then increased the dose to 6 tablets twice a day. He had no adverse side effects and felt more energetic. MRI showed the brain tumor mass decreased by 50% in size. Clinically there were decreased breath sounds but good tidal volume. The palpable lymph nodes decreased by approximately 50% in size. He remained stable for 3 years after taking the drug. He then stopped the medication for personal reasons and two weeks later died.

Thus overall, following initiation of the 5-FU oral nanoparticle therapy there was an objective measure of response to the therapy, a low level of side effects from a drug that is normally toxic to patients, and the patient lived well beyond the usual expectation for someone with his stage of metastatic testicular cancer.

In accordance with this invention, various other nanoparticulate formulations systems of the type described herein can be used to effect bioavailability, including lymphatic delivery under the conditions and parameters described above. In addition, other chemotherapeutic agents may be formulated into (encapsulated by) the nanospheres of the present invention for oral delivery and may be used as appropriate for the particular cancer to be treated.

Example 12

Nanosphere Formulation Encapsulating the Chemotherapeutic Agent Paclitaxel for Treatment of Metastatic Cancer in Humans and Other Mammals. Nanospheres are prepared encapsulating paclitaxel as follows. 2 gm PLGA (50:50), inherent viscosity .4 dl/gm, are dissolved in 50 ml dichloromethane. To this mixture is added 200mg paclitaxel

and the solution is blended at high speed with a Silverson homogenizer until homogeneous. The mixture is poured slowly into a beaker containing 400 ml water to which has been added 2% (w/v) PVA. The aqueous oil in water emulsion then is homogenized at high speed until light microscopy of a sample of the mixture shows uniform droplets of the required small size. The mixture is dried in a spray dryer and the dry powder is collected. 200 mg of the paclitaxel nanosphere powder are blended with 100 mg each of Carbopol 934-P and Methocel, and the combination is filled into gelatin capsules.

The resulting paclitaxel nanospheres may be used to treat orally animals and humans with cancer including breast cancer, ovarian cancer, lung cancer and other metastatic cancers against which paclitaxel has activity.

Example 13

Nanospheres for Oral Administration of Growth Hormone Releasing Factor (GRF) to Humans. This example demonstrates that oral administration of nanospheres of the present invention can be used to deliver therapeutic bioactive molecules *in vivo* in order to produce a biological response in humans. The prior art offers no guidance with respect to this therapeutic goal.

Nanospheres encapsulating GRF were prepared as described in Example 14, using mannitol as a protective agent. As shown by the data presented below, in the subjects who were orally administered the nanospheres, the growth hormone (GH) plasma levels gradually decreased, showing suppression of the short and long feed back loop, and providing evidence of drug absorption by these individuals. No one experienced adverse side effects. All the subjects showed a slight weight gain and some increase in upper body strength. They were followed weekly for 8 weeks without adverse effects from a single dose of the hormone. These data show that a polypeptide can be administered in a single oral dose of nanoparticles of the present invention and produce sustained biological effects of up to several weeks in humans.

Subject	Age Weeks	Weight kg	BP	Weeks	Subject	Age	Weight kg	BP	
(a)	27	117.4	110/68	0	(b)	33	95.24	80/56	0
		118.46	120/60	1			96.64	78/56	1
		119.56	100/60	2			97	74/48	2

		119.76	120/60	3	97.2	80/62	3
		118	110/60	4	98	77/60	4
		119	120/62	5	95.8	80/60	5
		119.5	124/70	6	99	82/58	6
		119.8	128/76	7	99.4	80/60	7
		120	122/64	8	100	84/58	8

	SubjectAge Weeks	Weight kg	BP	Weeks		SubjectAge Weeks	Weight kg	BP	
(c)	28	83	100/72	0	(d)	48	80	100/74	0
		85.3	90/66	1			81	98/68	1
		84.3	90/60	2			83	98/60	2
		84.9	80/60	3			83.3	100/58	3
		85	80/60	4			83.4	90/58	4
		85.5	90/62	5			83.4	100/60	5
		86	86/64	6			83.7	90/60	6
		87.2	87/65	7			84.6	90/60	7
		87	90/70	8			87	110/64	8

Growth Hormone (GH) Levels for Patients a, b, c, d

Week	a	B	C	d
0	1.7ug/mL	1.6 ug/mL	1.3 ug/mL	1.5 ug/mL
1	1.2 ug/mL	1.4 ug/mL	0.7 ug/mL	1.0 ug/mL
2	0.9 ug/mL	1.2 ug/mL	0.5 ug/mL	0.6 ug/mL
3	0.6 ug/mL	0.8 ug/mL	<0.4 ug/mL	0.5 ug/mL
4	0.5 ug/mL	0.7 ug/mL	<0.4 ug/mL	0.5 ug/mL
5	0.2 ug/mL	0.4 ug/mL	<0.4 ug/mL	0.4 ug/mL
6	<0.4 ug/mL	<0.4 ug/mL	<0.4 ug/mL	<0.4 ug/mL
8	<0.4 ug/mL	<0.4ug/mL	<0.4 ug/mL	<04 ug/mL

Nanospheres were made using a protein fragment (polypeptide) of growth hormone. Human Test Subjects (a)-(d) were orally administered a single dose of GRF nanospheres. The growth hormone (GH) blood level for each subject appeared suppressed after oral dosing. In particular, Subject (a) had an initial GH blood level of 1.7μg/mL, which was measured at less than 0.4 μg/mL after eight weeks. (Subject (a) received 25mg of the nanospheres.) Each of Subjects (b)-(d) received a 100mg dose, and an initial GH blood level for each (1.6, 1.3 and 1.5μg/mL, respectively) became undetectable after eight weeks. Suppression of GH levels is expected upon achievement of sufficient levels of

GRF in the plasma. Each Subject reported more strength and endurance post dosage. A weight gain was also observed for each Subject, but bodybuilding activities may be a contributing factor.

Below is the method used for production of these nanospheres.

Example 14

GRF Nanosphere Preparation. GRF Nanospheres as described above in Example 13were formulated as follows:

Ingredient	Amount
1. KH_2PO_4 pH 4.0 with glacial acetic acid	200 mL
2. Beta Cyclodextrin	1,000 mg
3. Polyvinyl Alcohol	100 mg
4. Mannitol USP	200 mg
5. Resomer 504 PLGA 50:50	1,000 mg
6. GRF (Protein fragment)	1,000 mg
7. Isopropyl Alcohol	10 mL

Cyclodextrin and GRF were added to the 200mL of acidified phosphate buffer and stirred by the Silverson® method using medium speed. Next the PVA was added. In a separate flask the Resomer was solvated using a magnetic stirring bar and dichloromethane. When the polymer was completely dispersed, the protein cyclodextrin mixture was added and blended at a high speed. Isopropyl alcohol was added to the blended mixture. Using a Buchi laboratory spray dryer, the entire flask of liquid material was spray dried and collected in the cyclone collection flask. Scanning electron microscopy showed nanosphere formation

Example 15

Alternative Procedure for Encapsulating Insulin in Biodegradable Nanoparticles.

Ingredient	mg or ml	wt %
1. Powered Insulin Novo Nordisk	500 mg	11.36%
2. Phospholipon 90H Lot # 22000900	400 mg	9.09%
3. Dextran Sulfate Lot# 60H179	500 mg	11.36%

4. Polyvinyl Alcohol Lot# 444024	500 mg	11.36%
5. KH_2PO_4 @ pH 6.8; 50 mM	300 mL	0%
6. Poly (3-hydroxybutyrate-co-hydroxyvalerate) (80:20 ratio; # 404838)	2,000 mg	45.45%
7. Poly (d,l-Lactide-co-Glycolide) # 411784	500 mg	11.36%
8. Dichloromethane	50 mL	0%
9. Acetone	50 mL	0%
	4,400 mg	100.00%

Procedure

1. Into 300 mL of KH_2PO_4 pH 6.8 was added PVA, with a Silverson® high shear blender. Following dispersion of the PVA in the buffer the insulin powder was blended into the fluid. After compete admixture Dextran and Phospholipon 9OH were added. This mixture was blended at medium speed until complete dispersion.
2. The 2 polymers #6+7 were blended separately with dichloromethane and acetone using a magnetic stirring bar.
3. As soon as the polymers were clear in the organic phase, they were poured into the buffer and blended at high speed until there was a heavy milky appearance.
4. Agitation in the Silverson® was carried out for a period of 5-7 minutes.
5. When there was evidence of sphere formation the solution was poured into a Pyrex® pan at 60oC for a period of 24 hours. When the film was dry it was removed and reduced in size by using a mortar and pestle and then passing the particles through a 25-26 mesh screen. The theoretical weight was checked against the actual weight.

Theoretical Weight = 4,400 mg Actual Weight = 3,680 mg

Yield = 83.6%

Example 16

Improved insulin nanosphere formulation. In an effort to obtain a more realistic dosage formulation and one which would produce more consistent results in animals the following nanosphere formulation was developed and then tested on diabetic rats.

Ingredient	mg	%
1. Eudragit RS 30 #0440236013	1,000 mg	39.22%
2. Phospholipon 90H Lot # 22000900	500 mg	19.61%

3. Beta Cyclodextrin Lot #D-196345	1,000 mg	39.22%
4. Insulin Powder	50 mg	1.96%
5. Ethyl Alcohol	50 mL	0.00%
	2,550 mg	100.00%

1. Insulin powder was placed into a beaker with cyclodextrin then phospholipids were added. This was placed into a beaker with aqueous Eudragit RS~50mL.
2. The mixture was stirred for 2 hours with a magnetic bar.
3. After two hours of stirring, the material was somewhat viscous. It was discharged into a Pyrex® pan and heated at 60°C for 3 hours after which it appeared dry and was removed easily by razor blade. The material was then screened to even size.

Theo Weight = 2,550 mg Actual Weight = 3,640 mg

The material was administered to streptozocin-induced diabetic rats and blood levels of glucose were monitored.

BLOOD GLUCOSE LEVELS
mg/dL
ORAL INSULIN NANOSPHERES

TIME	CONTROL	RAT 1	RAT 2	RAT 3	RAT 4	RAT 5
Baseline	67	75	67	69	70	85
Diabetes Onset & Dosing	74	301	307	445	415	420
6 HOUR	69	241	120	121	140	115
24 HOUR	70	312	85	56	75	45
48 HOUR	85	125	72	75	80	47
96 HOUR	75	100	65	110	85	58

The formulation of this example showed significantly improved suppression of glucose levels relative to the prior art and extension of the effect to at least 96 hr. post dose. In the present example the insulin weight percentage was reduced relative to the polymers and cyclodextrin.

* * *

While the principles of this invention have been described in connection with specific embodiments, it should be understood clearly that these descriptions are provided only by way of example and are not intended to limit, in any way, the scope of this invention. For instance, the present invention can be applied more specifically to delivery platforms for a variety of hormones or hormone factors, in addition to the growth hormone releasing factor discussed above. Other benefits, features and advantages will become apparent from the claims hereafter, with the scope thereof determined by the reasonable equivalents, as would be understood by those skilled in the art made aware of this invention.

WHAT IS CLAIMED:

1. A method of using the structural delivery component of a nanoparticulate formulation to modify the plasma concentration of a therapeutic agent, said method comprising:

providing a nanoparticulate formulation comprising a therapeutic agent and a structural delivery component, a lactide-glycolide co-polymer in an amount sufficient to achieve a therapeutic plasma concentration of said agent and sustain said concentration over time.

2. The method of claim 1 wherein said co-polymer and said therapeutic agent are present in said formulation at about the same weight percentages.

3. The method of claim 2 wherein said therapeutic agent is selected from the group consisting of heparin and insulin.

4. The method of claim 1 wherein said formulation includes beta-cyclodextrin.

5. The method of claim 1 wherein said formulation includes polyvinyl alcohol.

6. The method of claim 1 wherein said formulation further includes a bioadhesive adjuvant.

7. A controlled release oral dosage formulation comprising a macromolecular therapeutic agent, a lactide-glycolide co-polymer and beta-cyclodextrin, said agent and co-polymer present in said formulation at about the same weight percentages.

8. The oral dosage formulation of claim 7 wherein said therapeutic agent is selected from the group consisting of heparin and insulin.

9. The oral dosage formulation of claim 8 further including a surfactant.

10. The oral dosage formulation of claim 9 wherein said surfactant is polyvinyl alcohol.

11. The oral dosage formulation of claim 7 further including a bioadhesive adjuvant.

12. A method of using a nanoparticulate formulation to enhance lymphatic up-take of a therapeutic agent, said method comprising:

providing a nanoparticulate formulation comprising a lactide-glycolide co-polymer and a therapeutic agent; and

oral administration of said formulation.

13. The method of claim 12 wherein said nanoparticulates are dimensioned between about 100 nanometers and about 2 micrometers.

14. The method of claim 13 wherein said agent is a chemotherapeutic agent.

15. The method of claim 14 wherein said chemotherapeutic agent is 5-fluorouracil.

PHOTOMICROGRAPH OF NANOSPHERES AND THEIR PASSAGE THROUGH LYMPHATIC DUCTS TO THE LYMPH NODE

Nanospheres Stained With Fluorescein

Figure 1A

PHOTOMICROGRAPH OF NANOSPHERES AND THEIR PASSAGE INTO THE LYMPH NODE

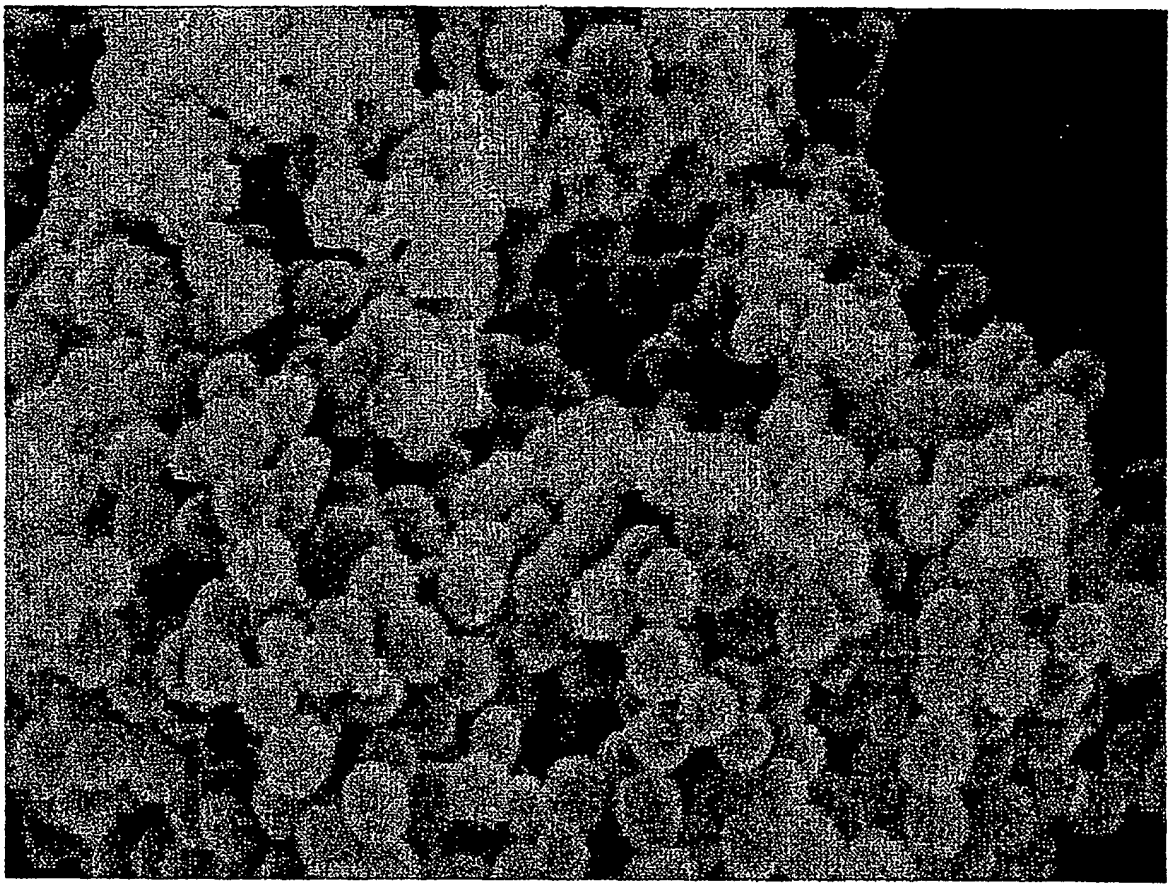
Figure 1B

PHOTOMICROGRAPH OF NANOSPHERES AND THEIR POSITION OF ADHERING TO THE INTESTINAL WALL

Nanospheres Stained With Fluorescein

Figure 1C

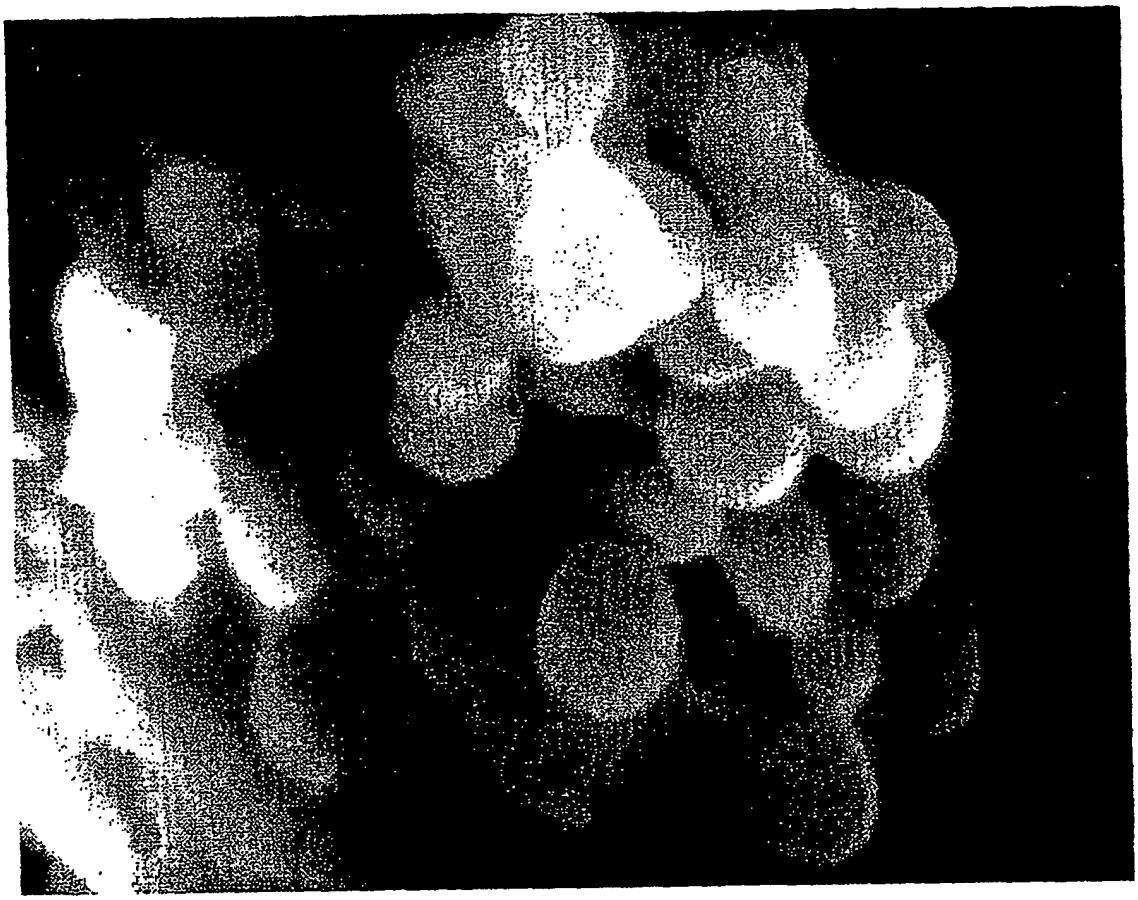
HEPARIN NANOSPHERES 600-800nm



25 KV@6,000 1.0u

Figure 2A

HEPARIN NANOSPHERES 600-800nm



15.1KV @13,000 1.0u

Figure 2B

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,117,455 A (TAKADA et al) 12 September 2000, see entire document.	1-15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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